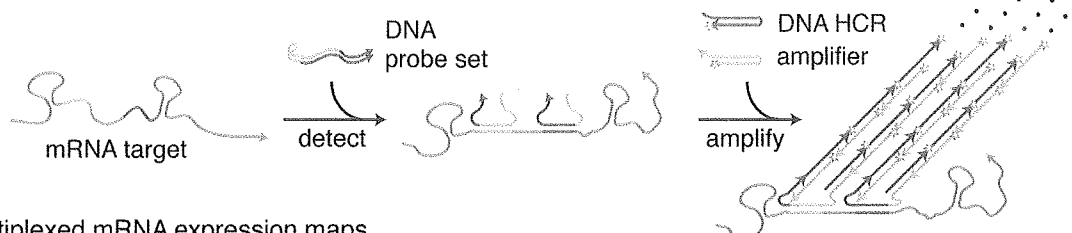


## Multiplexed *In Situ* Hybridization Using Hybridization Chain Reaction

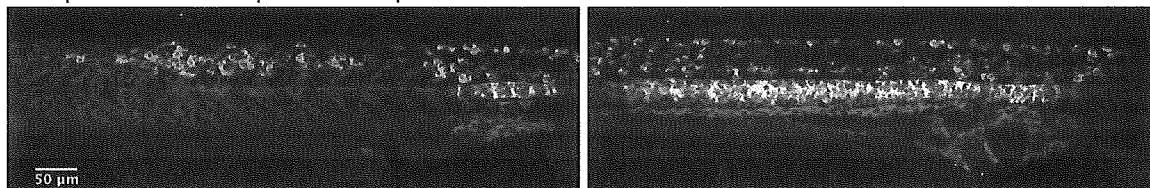
Harry M.T. Choi,<sup>1</sup> Victor A. Beck,<sup>1</sup> and Niles A. Pierce<sup>1,2</sup>

CHOI *ET AL.*<sup>1</sup> DESCRIBE PROGRAMMABLE *IN SITU* AMPLIFIERS based on the mechanism of hybridization chain reaction (HCR) that enable simultaneous mapping of multiple target mRNAs within whole-mount zebrafish embryos. In contrast to traditional *in situ* hybridization approaches, HCR *in situ* amplification is performed for all target mRNAs simultaneously, so the experimental timeline is independent of the number of target mRNAs. Using HCR, probes complementary to mRNA targets trigger chain reactions in which metastable fluorophore-labeled hairpins self-assemble into tethered fluorescent amplification polymers *in situ* (Fig. 1), enabling straightforward multiplexing for up to five target mRNAs at a time, deep sample penetration, high signal-to-background, and subcellular signal localization. The new DNA HCR technology<sup>1</sup> improves on the original RNA HCR technology<sup>2</sup> to dramatically increase signal gain, reduce reagent cost, and increase reagent durability. DNA HCR kits containing probe sets, amplifiers, and buffers can be purchased from the nonprofit academic resource ([www.molecularinstruments.org](http://www.molecularinstruments.org)). Advice and technical support can be obtained by emailing [support@molecularinstruments.org](mailto:support@molecularinstruments.org)

### Programmable molecular amplifiers



### Multiplexed mRNA expression maps



**FIG. 1.** Multiplexed mapping of mRNA expression within intact zebrafish embryos using next-generation DNA hybridization chain reaction (HCR). [Image from Choi *et al.* (2014); <http://pubs.acs.org/doi/abs/10.1021/nn405717p>; used with permission from the American Chemical Society.] Color image is available online at [www.liebertpub.com/zeb](http://www.liebertpub.com/zeb)

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# Zebrafish

VOLUME 11

NUMBER 5

OCTOBER 2014

## RESEARCH ARTICLES

- Derivation and Long-Term Culture of an Embryonic Stem Cell-Like Line from Zebrafish Blastomeres Under Feeder-Free Condition** 407  
*Sing Yee Ho, Crystal Wei Pin Goh, Jen Yang Gan, Youn Sing Lee, Millie Kuen Kuen Lam, Ni Hong, Yunhan Hong, Woon Khiong Chan, and Alexander Chong Shu-Chien*
- The Involvement of Cholesterol in Sepsis and Tolerance to Lipopolysaccharide Highlighted by the Transcriptome Analysis of Zebrafish (*Danio rerio*)** 421  
*Sonia Dios, Pablo Balseiro, Maria M. Costa, Alejandro Romero, Sebastián Boltaña, Nerea Roher, Simon Mackenzie, Antonio Figueras, and Beatriz Novoa*
- Zebrafish Embryo Model of *Bartonella henselae* Infection** 434  
*Amorce Lima, Byeong J. Cha, Jahanshah Amin, Lisa K. Smith, and Burt Anderson*
- Hemodynamics and Ventricular Function in a Zebrafish Model of Injury and Repair** 447  
*Juhyun Lee, Hung Cao, Bong Jin Kang, Nelson Jen, Fei Yu, Chia-An Lee, Peng Fei, Jinhyoung Park, Shadi Bohloul, Lian Lash-Rosenberg, K. Kirk Shung, and Tzung K. Hsiai*
- High-Throughput Analysis of Behavior in Zebrafish Larvae: Effects of Feeding** 455  
*Danielle Clift, Holly Richendrfer, Robert J. Thorn, Ruth M. Colwill, and Robbert Creton*
- Estrogen Regulation of microRNAs, Target Genes, and microRNA Expression Associated with Vitellogenesis in the Zebrafish** 462  
*Amit Cohen and Yoav Smith*
- Heterochromatin Polymorphism and Physical Mapping of 5S and 18S Ribosomal DNA in Four Populations of *Hypostomus strigaticeps* (Regan, 1907) from the Paraná River Basin, Brazil: Evolutionary and Environmental Correlation** 479  
*Lucas Baumgärtner, Leonardo Marcel Paiz, Cláudio Henrique Zawadzki, Vladimir Pavan Margarido, and Ana Luíza de Brito Portela Castro*

## TECHNOFISH

- Multiplexed *In Situ* Hybridization Using Hybridization Chain Reaction** 488  
*Harry M.T. Choi, Victor A. Beck, and Niles A. Pierce*

## FISH HAUS

- Tolerance and Efficacy of Emamectin Benzoate and Ivermectin for the Treatment of *Pseudocapillaria tomentosa* in Laboratory Zebrafish (*Danio rerio*)** 490  
*Chereen Collymore, Virginia Watral, Julie R. White, Michael E. Colvin, Skye Rasmussen, Ravi J. Tolwani, and Michael L. Kent*
- Correction** 498

**Cover Art:** Pan-cytokeratin positive cells arising from zebrafish embryonic stem cells. The pluripotent embryonic stem cells were maintained in a feeder free culture system.

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